REMARKS

Claims 1, 3 – 5, 7, 12 – 15 and 17 are pending in the application. Claim 1 has been amended to correct an obvious typographical error. Claims 130-136 have been added. No new matter has been added by virtue of these amendments; support therefore can be found in throughout the specification and original claims of the application. For example, claims 130 and 131 are supported on page 10, lines 21-23; claim 132 is supported on page 11, lines 3-4; claim 133 is supported on page 18, lines 20-24; claim 134 is supported on page 57, line 31; claim 135 is supported on page 56, line 26. Claim 136 is supported on page 46, beginning at line 26. Claims 2 – 4, 6, 8 – 11, 16, 19, 20, 22 - 129 were previously cancelled.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Rejection of Claims 1, 3 – 5, 7, 12 – 15 and 17 Under 35 USC 103(a)

The Examiner has maintained the rejection to claims 1, 3 – 5, 7 12 – 15 and 17 under 35 USC 103(a) as being unpatentable over Dominguez et al. (J of Immunological Methods, 1998, 220: 115 – 221) in view of Hooper et al. (USPN 6, 451, 309; the '309 reference herein). Applicants respectfully traverse the rejection.

The instant claims recite a method comprising incubating a mixture comprising at least one cell, a labeled invasin that encodes a detectable label, wherein the labeled invasin is a virus, and a candidate agent under conditions wherein the labeled invasin can invade the cell; and detecting the detectable label within the cell, wherein a decrease of detectable label in the cell due to the candidate agent indicates that the candidate agent decreases invasion of the cell by the invasin. Further dependent claims have been added to specifically recite that the assay is a neutralization assay, wherein the results obtained

by the method correlate with viral lethality *in vivo*; wherein the assay is a high throughput assay; wherein the method further comprises invasion of a cell by an invasin can be compared and quantitated through use of a standard curve; and the r^2 of the standard curve is >0.9; and that the assay is carried out in a plate comprising 96 wells, to point out further aspects of the invention disclosed, but not previously explicitly claimed.

In the prior response, Applicant asserted that it would not be obvious to combine the methods of Dominguez and Hopper as asserted in the Office Action. Applicant maintains this position.

In response to Applicant's arguments, the Office Action states that

> the Office is aware of the limitations of the Dominguez reference concerning the missing candidate agent aspect. However, the obviousness rejection of record sets forth that it would have been obvious to use the vaccinia-GFP construct of Dominguez to test the infectivity of cells in the presence of Hooper's monoclonal antibodies to determine whether the antibodies are effective agents that inhibit vaccinia virus infectivity… ***Hooper, [notes that] neutralization tests were not always predictive of protective efficacy in mice upon challenge***. (Office Action, p.4, emphasis added).

In column 2, lines 14-19, Hooper states:

> To our surprise, the ability of the MAbs to inhibit plaque formation by vaccinia virus, a standard assay of virus neutralization, ***did not always predict their protective efficacy***. ***Moreover, the monoclonal antibodies differed in their ability to provide protection depending on the challenge model.*** (emphasis added)

Hooper concludes that the ability of an antibody to provide protection in a challenge model is dependent on the antibody used, ***not the results from the neutralization assay used***. By providing antibodies that are protective, and later characterizing their binding to a specific epitope, Hooper cannot be understood to provide motivation to test the antibodies in an *in vitro* assay, such as that provided by Dominguez, as their activity is already known. Moreover, ***Hooper teaches that in vitro assays are not reliable***.

Further, Dominguez was published two years before the priority date of the Hooper application. Based on Hooper's discussion of the unpredictability of *in vitro* testing methods, it could not have been obvious to use the method of Dominguez to provide an assay such as that instantly claimed. ***Hooper teaches the use of the PRNT assay for analyzing the activity of other antibodies that bind the same epitopes as the antibodies provided therein*** (column 6, lines 14-16). Provided with this well established method, there can be no motivation to modify Dominguez to use the antibodies of Hooper, or to modify Hooper to use an alternate, non-established assay method to try to predict in vivo activity when in vitro methods are known to be unreliable.

The reference cited by the Office Action indicates the difficulty of developing an assay that is predictive of efficacy in mice upon challenge. That difficulty is confirmed by the instant application which demonstrates substantial variability in results depending on the laboratory that performed the infectivity assay and the specific cell lines used (see, e.g., page 50, Table 3). Having a validated method, one of skill in the art would not look to alternative methods.

Even as late as 2007, the PRNT assay was still the gold standard for determining serum antibody levels to predict protection from subsequent infection, despite the problems of the assay. Applicant provides herewith a copy of the "Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses" published by the World Health Organization in 2007 (hereinafter referred to as the Guidelines). The Preface and Background section (page vii) discusses the use of the PRNT assay.

> In an attempt to make inter-laboratory information more directly comparable, WHO and PDVI initiated a program to harmonize the procedures used for the plaque-reduction neutralization test (PRNT). The PRNT is the most common assay used to measure neutralizing antibody. The presence of antibody is believed to be most relevant for determining protective anti-DEN virus (DENV) immunity. While other neutralizing antibody assays are being considered for use in large scale vaccine field trials, ***the PRNT is still considered to be the laboratory standard against which other neutralizing antibody assays***

> ***should be compared*** (Martin et al., 2006; Vorndam and Beltran, 2002). [emphasis added]

On page 1 of the Guidelines, it is noted that

> The virus PRNT remains the most widely accepted approach to measuring virus-neutralizing and protective antibodies. Newer assays measuring virusneutralizing antibodies are being developed and will be briefly discussed later in this document.

Page 5 of the Guidelines discuss other tests, and their shortcomings:

> A variety of serological tests have been used to measure anti-flaviviral antibody. These tests include the hemagglutination-inhibition test, complement fixation test, fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA), and PRNT. Each of these tests measures different antibody activities. Only the PRNT measures the biological parameter of *in vitro* virus neutralization and is the most serologically virus-specific test among flaviviruses, and serotype-specific test among dengue viruses, correlating well to serum levels of protection from virus infection. ***Newer tests measuring virus neutralization are being developed, but PRNT remains the laboratory standard against which these tests will need to be validated.*** (page 5, emphasis added)

Therefore, despite the difficulties and problems of the PRNT test, no test discussed in the Guidelines had been developed by 2007 to supplant the PRNT assay or validated against the PRNT assay. Therefore, one would not expect to be successful in developing an assay that could predict viral lethality *in vivo* based on an *in vitro* test as so many previously developed tests had failed.

The steps involved in the PRNT assay are discussed on page 6 of the Guidelines. As noted the method

> The virus PRNT assay was first described in the 1950s, and was later adapted to DENV (Russell et al., 1967). The basic design of the PRNT allows for virus-antibody interaction to occur in a test tube or microtiter plate, and then measuring antibody effects on viral infectivity by plating the mixture on virus-susceptible cells. The cells are overlaid with a semi-solid media that restricts spread of progeny virus. Each virus that

> initiates a productive infection produces a localized area of infection (a plaque), that can be detected in a variety of ways. Plaques are counted and compared back to the starting concentration of virus to determine the percent reduction in total virus infectivity. In the PRNT, the serum specimen being tested is usually subjected to serial dilutions prior to mixing with a standardized amount of virus. The concentration of virus is held constant such that, when added to susceptible cells and overlaid with semi-solid media, individual plaques can be discerned and counted. In this way, PRNT end-point titers can be calculated for each serum specimen at any selected percent reduction of virus activity. ***A disadvantage of the PRNT is that it is labour intensive and therefore not readily amenable to high throughput, making it difficult to use for large-scale surveillance and vaccine trials***. (emphasis added)

On page 6, the Guidelines note that the size dish that can be used for the assay is limited by the ability too discern individual plaques and minimize plaque overlap. The need to discern individual plaques prevents the method from being performed in plates having at least 96 wells which are routinely use for high throughput assay methods.

> The challenge virus dose and the number of repeats tested for each dilution (serum or virus) are key factors for achieving accurate measurements. The challenge dose should be modified based on the surface area of the cell monolayer (e.g., 6-well versus 24-well plates), to get readily discernable plaques and minimize plaque overlap. ***The target number of plaques per well can vary by virus strain, however 40-60 pfu per 35 mm dish should permit accurate titrations while minimizing plaque-overlap***. Plaque overlap results from crowding of plaques in an individual well. Comparing plaque counts in the test system versus input virus that has been "back-titrated" is the most acceptable way to rule out plaque-overlap. In order to reach an acceptable precision for the plaque counts, it is recommended that at least three repeat wells for a challenge dose of 50 PFU/reaction or less be used.

As noted, to achieve sufficient numbers readily discernable plaques prevent modification of the PRNT assay, or any other assay relying on plaque counting, from being performed in small wells (e.g., those of a 96-well or 384-well plate), and require replicate wells at multiple doses making the assay even more cumbersome.

The present invention is directed to the development of a novel assay to measure protection of cells against virus invasion; a novel neutralization assay. ***As taught in the specification, the method as claimed is the only validated alternative method to the classical PRNT Assay.*** As taught in the specification (e.g. page 46, beginning at line 26) ***results obtained using beta-gal in the instantly claimed method, are comparable to results obtained with the classic PRNT vaccinia neutralization assays.*** Moreover, as taught in the specification, the high throughput technology makes the claimed method highly sensitive (e.g. page 47, beginning at line 14), easier to conduct (even with small volumes), faster, and easy to transfer to other laboratories (e.g. page 39, beginning at line 39).

The usefulness of the instantly claimed assay and its adaptability to a high-throughput method has been further demonstrated in a publication of the inventor of the instantly claimed method in conjunction with collaborators (Kennedy et al., Statistical approach to estimate vaccinia-specific neutralizing antibody titers using a high-throughput assay. *Clin. Vac. Immunol.* 16:1105-12, 2009, copy enclosed) demonstrating the ease with which the method claimed can be converted to a high throughput screening method.

In yet another publication by the inventor, the method provided herein was demonstrated to predict lethality in vaccinia virus infected mice (Zaitseva et al., Application of bioluminescence imaging to the prediction of lethality in vaccinia virus-infected mice. *J. Virol.*, 83:10437-47, copy enclosed). It is clear from the Guidelines discussed above that the development of an assay to determine the protection afforded by antibodies or other agents could only be achieved with the PRNT assay and no other assays discussed therein.

The MPEP discusses the requirement for a reasonable expectation of success. As noted above and quoted from the Guidelines, a number of assays have been developed to different antibody activities, but not virus neutralization.

2143.02 Reasonable Expectation of Success Is Required [R-6]

> A rationale to support a conclusion that a claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 USPQ2d 1385, 1395 (2007); *Sakraida v. AG Pro, Inc.*, 425 U.S. 273, 282, 189 USPQ 449, 453 (1976); *Anderson's-Black Rock, Inc. v. Pavement Salvage Co.*, 396 U.S. 57, 62-63, 163 USPQ 673, 675 (1969); *Great Atlantic & P. Tea Co. v. Supermarket Equipment Corp.*, 340 U.S. 147, 152, 87 USPQ 303, 306 (1950).
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> Obviousness does not require absolute predictability, however, at least some degree of predictability is required. ***Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness***. In re Rinehart, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976) (Claims directed to a method for the commercial scale production of polyesters in the presence of a solvent at superatmospheric pressure were rejected as obvious over a reference which taught the claimed method at atmospheric pressure in view of a reference which taught the claimed process except for the presence of a solvent. The court reversed, finding there was no reasonable expectation that a process combining the prior art steps could be successfully scaled up in view of unchallenged evidence showing that the prior art processes individually could not be commercially scaled up successfully.).

Applicant notes that the PRNT assay was developed in the 1950s and is admitted to be a cumbersome and difficult assay. As of 2007, the WHO did not recognize an assay to replace the PRNT assay. Therefore, success cannot be expected in combining references to arrive at an assay that could be validated against the PRNT assay while providing an assay that could be used as a high throughput assay. Particularly in view of references that do not teach assays to determine if an agent could indicates that the candidate agent decreases invasion of the cell by an invasion.

The Dominguez reference teaches the construction of recombinant vaccinia expressing GFP for detection of cells by flow cytometry. However, the construct as taught by Dominguez, is merely used "as an infection tag" and "is useful for studying tropism." There can be no suggestion to use the method to provide a quantitative assay, or that the method could be used to provide an assay that is accurately predictive of results from an *in vivo* challenge assay or could be validated against a PRNT assay.

The methods of the present Application provide a prediction of virus lethality, as now claimed, that is not possible with the methods taught by either Dominguez or Hooper either alone, or when taken alone or in combination, particularly in view of the lack of predictability of in vitro assays as taught by Hooper.

Dominguez merely teaches "the usefulness of GFP as an infection marker" (p. 120), not as a marker that would be useful in neutralization assays that are predictive of virus lethality as claimed. In determining tropism, there can be no motivation to include a composition in the assay that would modulate binding or invasion of the virus into the cell as it would interfere with the ability to determine the tropism of the virus.

Further, as pointed out by the Examiner, the '309 reference only teaches the production and potential activity of the monoclonal antibodies against vaccinia. The '309 reference does not teach or suggest a reporter-based- assay (for example, B-galactosidase vaccinia virus or GFP-expressing vaccinia) to demonstrate the protective activity of their monoclonal antibodies.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejection of Claims 18 and 21 Under 35 USC 103(a)

The Examiner has maintained the rejection to claims 18 and 21 under 35 USC 103(a) as being unpatentable over Dominguez et al. (J of Immunological Methods, 1998, 220: 115 – 221) in view of Hooper et al. (USPN 6, 451, 309; the '309 reference herein) as applied to claims 1 and 17, above, and further in view of Englemayer et al. (The J of Immunology, 1999, 163: 6762 – 6768). Applicants respectfully traverse the rejection.

As set forth above, the combination of Dominguez and Hooper fail to teach the invention as claimed. The Englemayer reference does not cure the flaws of Dominguez and Hooper.

No combination of the cited art teaches the method as instantly claimed, in particular a method to measure protection of cells against virus invasion by measuring a decrease in invasion by a candidate agent. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Early consideration and allowance of the application are earnestly solicited.

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Respectfully submitted,

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